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SUMMARY

Counterimmunoelectrophoresis (CIE) has become a widely used test for the rapid identification of bacterial infections. It is less often used for identifying viral infections except for hepatitis B infections, in which CIE was the earliest test used for its detection. CIE testing has traditionally been performed using a barbital buffer as the electrolyte of choice. This is most likely due to the fact that barbital buffers were used for paper electrophoresis in serum protein studies for many years. The barbiturates used in these buffers have now become controlled substances due to their potential for drug abuse. The continued use of barbiturate buffers for electrophoresis then becomes an unsafe laboratory practice.

We report here the compositions of four non-barbiturate buffers, each appears to have the potential of replacing the barbital buffer in the CIE test. We used these buffers to rapidly detect 4 bacterial and one viral antigen in the CIE test. The sensitivity achieved with these non-barbital buffers with these five antigens was comparable to that achieved with the standard barbital buffer. Further evaluation of the use of non-barbiturate buffers in the CIE test are being conducted.

INTRODUCTION

Counterimmunoelectrophoresis (CIE) has traditionally been performed utilizing barbital buffers, a carryover from the serum protein electrophoresis technique used the past 4 decades. However, the barbiturates, with a potential for drug abuse, are now controlled chemicals. The use of barbital buffers, therefore, creates a potential laboratory hazard which non-barbital alternative(s) would eliminate. A recent description of two non-barbiturate buffers for electrophoresis of serum proteins on cellulose membrane suggested their usefulness for CIE testing (1).

This report compares CIE identification of 1 viral and 4 bacterial antigens using 4 non-barbiturate buffers and 1 barbiturate buffer.

MATERIALS AND METHODS

Agarose: Indubiose A45, lot #3162 (Fisher Scientific Co)

Antigens: Three spinal fluids from patients with cultures positive for H. influenzae type b, (supplied by Dr. John Sipple, Naval Biomedical Labs, Oakland); meningococcus group A antigen, purified polysaccharide (Lot #M1072, Merieux Institute, Lyon, France); pneumococcus antigen, (vaccine polysaccharide, Pneumocoque Tetradecavalent Lot #5088-AOUT 80, Merieux Institute, Lyon, France) Streptococcus group A, (In-House produced extract by autoclaving (2).

Antisera: Meningococcus group A (Lot #1-80, a gift of the Environmental Preventive Medicine Unit #5, San Diego); H. influenzae type b (kindly supplied by CDC, Atlanta); pneumococcus (OMNI antiserum, Lot #49-79, Statens Seruminstitut, Copenhagen) and streptococcus group A (Lot #K8291, Wellcome Reagent Ltd., England).

Buffers: Buffer A, 13.0 gr Tris; 5.4 g Tricine; 0.424 gr calcium lactate; 0.8 g sodium azide per liter distilled water.

Buffer B. 8.0 g Tris; 3.75 g Tricine; 2.25 g sodium chloride, 0.75 g sodium salicylate per liter distilled water (1).

Buffer C. 7.5 g Tris; 10.0 gr sodium hippurate; 3.0 g hippuric acid per liter distilled water (1).

Buffer D. 8.0 g Tris; 3.75 Tricine g per liter distilled water.

Buffer E. Barbital Buffer (Lot #900M240 Kallestad Labs)

CIE Test: A 1% suspension of agarose was made in each of the 5 buffers. Twelve ml of each buffered suspension were pipetted onto separate lantern slides (80 x 100 mm) (3). The agarose was allowed to gel in a moist chamber at room temperature. Two parallel rows of wells, 3 mm in diameter, were cut 1.5 mm apart. Serial dilutions of antigen were placed in the wells nearest the cathode and undiluted antisera was placed in those wells nearest the anode. Electrophoresis with the appropriate buffers in the electrode vessels was for 60 minutes with 12-15 mA per slide. Examination for a precipitin reaction between the wells was made immediately after electrophoresis and after 24 hours.

RESULTS

The antigen-antibody precipitin reactions for the 5 antigens tested appeared similar in all 5 buffers and the clarity of the precipitin was comparable in all buffers. The reciprocal of the highest dilution of each antigen yielding a positive precipitin for each buffer system is shown in Table I. Except for pneumococcal antigen in buffer A and H. influenzae type b antigen in buffer C, the highest dilution of each antigen producing a positive test was similar for each of the non-barbital buffers and the barbital buffer.

TABLE I.

A Quantitative Study of Five Bacterial Antigens by CIE

Using Five Different Buffer Electrolytes

	ANTIGEN *				
Buffers	Strep A*	Pneumococcus	Meningococcus A	Adenovirus Type 4	Hemophilus Influenzae Type b
Buffer A	8 **	1024	512	8	8
Buffer B	16	4096	1024	8	4
Buffer C	16	4096	512	8	0
Buffer D	8	4096	1024	8	ND
Buffer E	8	4096	1024	8	8

* Antigens diluted $\log_2$ with physiological saline. Results represent the recriprocal of the higest dilution giving a precipitin reaction.

** Titer from 3 trial runs performed on different days.

ND - Not tested

DISCUSSION

Barbital buffers, long the standard for electrophoresis, are now controlled chemicals and their use in a laboratory creates a legal and administrative burden. Alternative buffers must demonstrate similar specificity and sensitivity.

This study compared results when 4 bacterial antigens and 1 viral antigen were identified by CIE using 4 non-barbital buffers and 1 barbital buffer. Non-barbital buffers B and D demonstrated comparable sensitivity to the barbital buffer for each antigen tested and non-barbital buffers A and C were comparable to the barbital buffer in 4 of 5 antigens tested.

The sensitivity achieved with these non-barbital buffers, in an admittedly small range of antigens, encourages their further evaluation. Buffers B and D particularly appear to be deserving of further testing. Studies with these buffers on a broader range of bacterial and viral antigens as well as on other parameters (pH, molarity, endosmosis, color distortion, storability, current stability, agarose concentration, time for the reaction to optimize, etc) are currently being conducted.

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Counterimmunoelectrophoresis (CIE) and cellulose electrophoresis have traditionally been run by using barbital buffers as the electrolyte of choice. We compared results of CIE identification of 1 viral and 4 bacterial antigens using four non-barbiturate buffers and a barbital buffer. One of the non-barbiturate buffers gave results equal to the barbital buffer for all 5 antigen systems tested. These data show that buffers other than barbital buffers can be used in the CIE test to identify bacterial and viral antigens.

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